NCT05423418

A Phase 1 Randomized Study to Evaluate the Safety, Tolerability, and Immunogenicity of Ranging Doses of ALFQ Adjuvant in a Candidate HIV Vaccine Containing A244 and B.65321

January 22, 2024

STATISTICAL ANALYSIS PLAN
for
Protocol: RV 575

Study Title:
A Phase 1 Randomized Study to Evaluate the Safety, Tolerability, and Immunogenicity of Ranging Doses of ALFQ Adjuvant in a Candidate HIV Vaccine Containing A244 and B.65321

RV575
IND #: 28352
Version 2.1

DATE: 22 January 2024

SAP Revision Histor

Revision Date	Analyst	Revisions/Justification
01Feb2023	Misti Paudel/Allahna Esber	Additional sensitivity analyses due to unanticipated vaccinations occurring out of study window
21 December 2023	Adam Yates/Tyler Hamby	Detailing interim Analysis plans for Humoral Immunity and dose response
11 January 2024	Adam Yates/Tyler Hamby	Finalized edits and Added CONSORT example diagram
18 January 2024	Adam Yates/Tyler Hamby	Clarified Interim analysis specifications for cellular response

Trial Initiation Date: first participant screened 08 Sept 2022
Trial Completion Date: last participant visit 29 Oct 2024

STUDY TITLE

Protocol Number Code:	**RV 575**
Development Phase:	Phase I
Products:	A244, B.63521, and ALFQ
Form/Route:	IM Injection
Indication Studied:	Preventative HIV vaccine
Sponsor:	The Surgeon General, Department of the Army
Clinical Trial Initiation Date:	
Clinical Trial Completion Date:	
Date of Analysis Plan:	11 January 2024
Version Number:	2.1

This study was performed in compliance with Good Clinical Practice.

Signatures

I give my approval for the attached SAP entitled "A Phase 1 Randomized Study to Evaluate the Safety, Tolerability, and Immunogenicity of Ranging Doses of ALFQ Adjuvant in a Candidate HIV Vaccine Containing A244 and B.65321" dated 21 December 2023

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Abbreviations

Abbreviation or Specialist Term	Explanation
3D-PHAD®	Synthetic monophosphoryl lipid A
AAE	Acquired angioedema
Ab	Antibody
ADCC	Antibody-Dependent Cell-Mediated Cytotoxicity
ADCP	Antibody-Dependent Cell-Mediated Phagocytosis
AE	Adverse event
AESIs	Adverse events of special interest
AIDS	Acquired immunodeficiency disease syndrome
AHFG	Aluminum Hydroxide Fluid Gel
ALF	Army Liposome Formulation
ALFQ	ALF mixed with the saponin QS21
AlOH	Aluminum hydroxide
ALT	Alanine aminotransferase
ATO	Army Technology Objective
AUC	Area under the curve
CD4	Cluster of Differentiation
CFSE	Carboxyfluorescein succinimidyl ester, a fluorescent cell staining dye
CRFs	Case Report Forms
CRR	Continuing review report
CTC	WRAIR Clinical Trials Center
DAIDS	Division of AIDS
DODI	Department of Defense Instruction
eCRF	Electronic Case Report Forms
EDC	Electronic Data Capture
EDTA	Ethylenediaminetetraacetic acid
ELISPOT	Enzyme-Linked Immunospot
EOSI	Events of Special Interest
FDA	U.S. Food and Drug Administration
FSH	Follicle stimulating hormone
GBS	Guillain-Barré syndrome

Abbreviation or Specialist Term	Explanation
GCLP	Good Clinical Laboratory Practices
GCP	Good Clinical Practices
HAE	Hereditary angioedema
HBsAg	Hepatitis B surface antigen
HIV-1	Human Immunodeficiency Virus 1
HRPO	Human Research Protections Office
HRT	Hormonal replacement therapy
HSPB	Human Subjects Protection Branch, WRAIR
IBs	Investigational brochures
ICH	International Conference on Harmonization
ICS	Intra-cellular cytokine staining
ID	Infectious disease
IEC	Independent Ethics Committee
IFNγ	Interferon gamma
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IL2	Interleukin 2
IM	Intramuscular
IND	Investigational New Drug
IP	Investigational product
IQR	Interquartile range
IRB	Institutional Review Board
IUD	Intrauterine device
IUS	Intrauterine hormone-releasing system
LPLV	Last Participant Last Visit
MAAE	Medically Attended Adverse Events
MHRP	U.S. Military HIV Research Program
MOP	Manual of Procedures
MPLA	Monophosphoryl lipid A
NAAT	Nucleic Acid Testing
NCO	Non-commissioned officer
NHP	Non-human primate

Abbreviation or Specialist Term	Explanation
NK	Natural Killer Cell
NKT	Natural Killer T cell
NSAIDs	Nonsteroidal anti-inflammatory drugs
OAE	Other significant adverse events
ORA	Office of Regulated Activities
ORP	Office of Research Protections, USAMRDC
PBMC	Peripheral blood mononuclear cell
PI	Principal Investigator
PIMMCs	Potentially Immune-Mediated Medical Conditions
PIN	Participant identification number
PSRT	Protocol Safety Review Team
PSSBO	Product Safety Surveillance Branch Office
PVG	Pharmacovigilance
RFADCC	Rapid and fluorometric ADCC
SAE	Serious adverse event
SD	Standard deviation
SGTP	Serum glutamic pyruvic transaminase
SMC	Safety Monitoring Committee
SOE	Schedule of Evaluations
SOP	Standard operating procedure
STI	Sexually transmitted infection
TOU	Test of understanding
UPIRTSO	Unanticipated problems involving risks to subjects or others
USAMRDC	United States Army Medical Research and Development Command
WRAIR	Walter Reed Army Institute of Research

1. PREFACE

The statistical analysis plan (SAP) for the Phase I "A Phase 1, Randomized, Double Blind Study to Evaluate the Safety, Tolerability, and Immunogenicity of Ranging Doses of Candidate Vaccine A244/B.63521 with ALFQ Adjuvant in Healthy Adults" expands upon the statistical considerations presented in the protocol. This document includes all planned analyses and associated justifications. It also includes sample tables, listings, and figures (TFLs) that will be populated for final deliverables.

2. INTRODUCTION

The purpose of this study is to optimize ALFQ dosing. This study is a Phase 1 three-arm randomized double-blind clinical trial. The primary outcomes for this study include safety assessments and secondary outcomes include cellular and humoral immune responses. Safety will be assessed through the frequency of the overall and specific post-vaccination reactions. Blood will be collected to assess humoral, cell-mediated, and innate immune responses.

Healthy adults not living with HIV, who are available for 12 months will be enrolled. A total of 60 participants will be enrolled in one of three arms, each comprised of 20 candidate vaccine recipients in an overall allocation of 1:1:1. A sentinel group will consist of the first 6 participants enrolled and will be used to assess safety prior to enrolling the remaining 54 participants. Each study arm will receive identical doses of A244 and B.63521 (300 micrograms of each protein). In addition, arm 1 will receive 200 micrograms of ALFQ. arm 2 will receive 100 micrograms of ALFQ; and arm 3 will receive 50 micrograms of ALFQ. The safety, reactogenicity, and immunogenicity will then be compared among the three arms to determine the optimal dose of ALFQ.

All vaccinations will be split into 2 half doses, which will be administered intramuscularly (IM) into the same deltoid muscle. Vaccinations will occur at months 0, 1, and 2. The second vaccination will be administered into the contralateral deltoid at study month 1 compared to the first vaccination at study month 0. The third vaccination at study month 2 will be administered into the same deltoid as the first vaccination at study month 0. Participants will be followed for 12 months following the last study vaccination.

2.1. Purpose of the Analysis

The purpose of these analyses is to assess the safety, tolerability (including reactogenicity), and immunogenicity of candidate vaccine A244/B.63521 (300 μg each) with ALFQ adjuvant at the 200 μg, 100 μg, and 50 μg doses.

3. STUDY OBJECTIVES AND ENDPOINTS

3.1. Study Objectives

3.1.1. Primary Objective

- To evaluate the safety and tolerability (including reactogenicity) of candidate vaccine A244/B.63521 (300 μg each) with ALFQ adjuvant at the 200 μg, 100 μg, and 50 μg doses

3.1.2. Secondary:

- To evaluate the effect of candidate vaccine A244/B.63521 (300 μg each) with ALFQ adjuvant at the 200 μg, 100 μg, and 50 μg doses on cellular, and humoral immune responses

3.1.3. Exploratory:

- To characterize B-cell functional specificities for each vaccination regimen
- To characterize innate immunity
- To assess the innate/gene expression induced across vaccination regimens
- To perform systems serology analyses

3.2. Endpoints/Outcome Measures

3.2.1. Safety Endpoints

- Evaluate the occurrence and severity of solicited local and systemic adverse events following candidate vaccine administration.
- Evaluate the occurrence, severity, and relationship to vaccination of unsolicited adverse events after candidate vaccine administration.
- Evaluate the occurrence of serious adverse events (SAEs), and new-onset medical conditions.
- Evaluate the occurrence of adverse events of special interest (AESIs) following candidate vaccine administration.

3.2.2. Immunogenicity endpoints

- Compare plasma IgG binding antibodies to gp120 in terms of magnitude, durability, and area under the curve among groups with differing ALFQ doses.
- Characterize and assess the magnitude of cell-mediated immune responses elicited across vaccination regimens including antigen -specific CD4 and CD8 T cell responses and polyfunctionality.

3.2.3. Exploratory endpoints

- Characterize plasma IgG and Immunoglobulin A (IgA) binding antibodies to HIV gp120, neutralizing antibodies, and non-neutralizing effector functions such as Antibody-Dependent Cell-Mediated Cytotoxicity (ADCC) and Antibody-Dependent Cell-Mediated Phagocytosis (ADCP) with emphasis on RV144 immune correlates of risk of HIV acquisition.
- Characterize B-cell functional specificities for each vaccination regimen by quantifying antigen-specific responses through B-cell Enzyme-Linked Immunospot (ELISPOT), phenotyping the magnitude and activation status of B cell subsets via flow cytometry and isolation of monoclonal antibodies from selected vaccine recipients.
- Characterize innate immunity through quantification of soluble chemokines and cytokines and assessing the phenotype and function of cellular innate immune subsets such as Natural Killer Cell (NK), NKT, and dendritic cells.
- Characterize effects of host genetic polymorphisms on immune responses and characterize differentially expressed genes.

Table S1: Immunology Assays

Humoral Assays	Serum or plasma	Function Measurement
ADCC, ADCP, and other non-neutralizing antibody functions	Frozen Plasma/ Serum	Measures lysis of HIV expressing targets mediated by HIV specific antibodies
HIV-specific binding	Frozen Plasma/ Serum	Binding antibody to vaccine antigens
HIV-specific neutralizing antibodies	Frozen Serum	Neutralizing activity against luciferase reporter gene expression
Cellular and Innate Assays	**Serum or plasma**	**Function Measurement**
Cellular response by cytokines such as IFN-y and IL2 after stimulation with HIV-specific antigens	Frozen PBMC	CD4+ and CD8+ antigen-specific response
Lymphocyte proliferation	Frozen PBMC	Characterize the function of proliferating cells in response to HIV antigens
B-cell ELISPOT	Frozen PBMC	Measures cytokine secretion from B cells in response to HIV antigens
Flow cytometry for innate immune cell phenotyping and a cytokine array assay	Frozen Plasma/ Serum	Phenotype NK and other innate cells and characterize the cytokines elicited by the different vaccine regimens
DNA Microarray: gene expression to vaccine antigens	Frozen PBMC	Host gene expression profile and signature to vaccine antigens
RNA sequencing: gene transcription to vaccine antigens	Frozen PBMC	Host gene transcription profile and signature to vaccine antigens

3.3. Study Definitions and Defined Variables

The baseline value will be defined as the last value obtained prior to the first vaccination. Individual antibody endpoint titers will be reported with values of C*2k, where k=0, 1, 2, etc.

and C may vary depending on the dilutions used for a given assay. Values below each assay's limit of detection will be imputed as one-half the limit of detection. For analysis, the geometric mean of replicates for each sample will be computed and used as the response for all subsequent calculations, where applicable.

Immune response over time will be assessed by the positive incremental area under the curve (AUC) based on a graph with the analysis value (e.g., log endpoint titer) on the y-axis and visit week on the x-axis.

4. INVESTIGATIONAL PLAN

4.1. Overall Study Design and Plan

This is a phase I randomized, double-blind clinical trial to optimize ALFQ dosing among healthy adults not living with HIV. A total of 60 participants will be enrolled to one of three arms (N=20 in each arm) with each arm receiving 300 micrograms of A244 and B.63521. In addition, arm 1 will receive 200 micrograms of ALFQ, arm 2 will receive 100 micrograms of ALFQ, and arm 3 will receive 50 micrograms of ALFQ. The safety, reactogenicity, and immunogenicity will then be compared among the three arms to determine the optimal dose of ALFQ.

All vaccinations will be split into 2 half doses which will both be administered intramuscularly (IM) into the same deltoid muscle. Vaccinations will occur at months 0, 1, and 2. The second vaccination will be administered into the contralateral deltoid at study month 1 compared to the first vaccination at study month 0. The third vaccination at study month 2 will be administered into the same deltoid as the first vaccination at study month 0. Participants will be followed for 12 months following the last study vaccination. Table S2 provides a schematic for the study design.

Table S2: Study Design

Number of participants (N) = 60		**Vaccination schedule in months (days)**		
	Number of participants (N)	**Month 0 (Day 1)**	**Month 1 (Day 29)**	**Month 2 (Day 57)**
Arm 1	20	300µg A244 300µg B.63521 200µg ALFQ	300µg A244 300µg B.63521 200µg ALFQ	300µg A244 300µg B.63521 200µg ALFQ
Arm 2	20	300µg A244 300µg B.63521 100µg ALFQ	300µg A244 300µg B.63521 100µg ALFQ	300µg A244 300µg B.63521 100 µg ALFQ
Arm 3	20	300µg A244 300µg B.63521 50µg ALFQ	300µg A244 300µg B.63521 50µg ALFQ	300µg A244 300µg B.63521 50µg ALFQ

The schedule of events is given in Table S3 below, for reference

Table S3: Schedule of Events (SOE)

Visit Number	0	1	2	3	4	5	6	7	8	9	10[2]/Exit 4	Phone Follow-up #1	Phone Follow-up #2
Visit Type	Screen	V1	Safety & Immun	V2	Safety & Immun	V3	Safety & Immun	Safety & Immun	Safety & Immun	Safety& Immun	Safety & Immun	MAAE follow-up	MAAE follow-up
Visit Day	-30 to -3	1	15	29	43	57	58-59	64	71	169	337	365	393
Window			± 3d	± 7d	± 3d	±7d		± 3d	± 3d	± 7d	± 7d	± 7d	± 7d
Visit Week		0	2	4	6	8	8	9	10	24	48	52	56
Clinical													
Briefing and Contact Information	X												
Informed Consent	X												
Test of Understanding	X												
Enrollment and Randomization		X											
Vaccination		X		X		X							
Vital Signs and Physical exam[1]	X	X	X	X	X	X	X	X	X	X	X		
Medical History & Concomitant Medications	X	X	X	X	X	X	X	X	X	X	X		
Adverse Event Documentation (AE, SAE, AESI)		X	X	X	X	X	X	X	X	X	X		

Visit Number	0	1	2	3	4	5	6	7	8	9	10[2]/Exit 4	Phone Follow-up #1	Phone Follow-up #2
Visit Type	Screen	V1	Safety & Immun	V2	Safety & Immun	V3	Safety & Immun	Safety & Immun	Safety & Immun	Safety& Immun	Safety & Immun	MAAE follow-up	MAAE follow-up
Visit Day	-30 to -3	1	15	29	43	57	58-59	64	71	169	337	365	393
Window			± 3d	± 7d	± 3d	±7d		± 3d	± 3d	± 7d	± 7d	± 7d	± 7d
Visit Week		0	2	4	6	8	8	9	10	24	48	52	56
Medically Attended Adverse Event (MAAE) Documentation[3]	X	X	X	X	X	X	X	X	X	X	X	X	X
Diary Card		X	X	X	X	X	X	X					
HIV Risk Counseling	X	X	X	X	X	X			X	X	X		
Pregnancy Test[7] & Pre/Post Counseling	X	X		X		X			X	X	X		
CBC w/ diff	4	4	4	4	4	4			4		4		
Creatinine, ALT	4	4	4	4	4	4			4		4		
Hepatitis B and Hepatitis C Serology	7												
HIV Testing	7		7		7				7	7	7		
Research[5]													
HIV Binding Antibody		SP	SP	SP	SP	SP			SP	SP	SP		

Visit Number	0	1	2	3	4	5	6	7	8	9	10[2]/Exit 4	Phone Follow-up #1	Phone Follow-up #2
Visit Type	Screen	V1	Safety & Immun	V2	Safety & Immun	V3	Safety & Immun	Safety & Immun	Safety & Immun	Safety& Immun	Safety & Immun	MAAE follow-up	MAAE follow-up
Visit Day	-30 to -3	1	15	29	43	57	58-59	64	71	169	337	365	393
Window			± 3d	± 7d	± 3d	±7d		± 3d	± 3d	± 7d	± 7d	± 7d	± 7d
Visit Week		0	2	4	6	8	8	9	10	24	48	52	56
HIV Neutralizing Antibody Assays		6	6	6	6	6			6	6	6		
Functional Antibody Assays		SP	SP	SP	SP	SP			SP	SP	SP		
B-Cell Analysis		17	17	17	17	17		17	17	17	17		
Multiparameter Flow Cytometry		17	17	17	17	17		17	17	17	17		
Innate Cell Analysis		17	17		17		17	17	17	17	17		
Transcriptomics		2.7	2.7	2.7	2.7	2.7	2.7	2.7	2.7				
Additional Immunogenicity Testing		42.5	8.5	8.5	8.5	8.5		17	25.5	34	34		
Daily Volume (mL)[6]	22	110.2	83.2	59.2	83.2	59.2	19.7	70.7	100.2	98	106		
Cumulative Volume (mL)	22	132.2	215.4	274.6	357.8	417	436.7	507.4	607.6	705.6	811.6		

Visit Number	0	1	2	3	4	5	6	7	8	9	10[2]/Exit 4	Phone Follow-up #1	Phone Follow-up #2
Visit Type	Screen	V1	Safety & Immun	V2	Safety & Immun	V3	Safety & Immun	Safety & Immun	Safety & Immun	Safety& Immun	Safety & Immun	MAAE follow-up	MAAE follow-up
Visit Day	-30 to -3	1	15	29	43	57	58-59	64	71	169	337	365	393
Window			± 3d	± 7d	± 3d	±7d		± 3d	± 3d	± 7d	± 7d	± 7d	± 7d
Visit Week		0	2	4	6	8	8	9	10	24	48	52	56
8-Week Cumulative Volume (mL)						395	414.7	375.2	475.4	98	106		

SP = Assay performed from stored plasma; no additional blood volume required

1 Full physical examination at screening (visit S1) and targeted physical examination at all other visits; Vitals to be collected both pre- and at about 30 minutes post-vaccination at Visit Day 1, 29, and 57.
2 Participants will be contacted for the unblinding visit after the clinical data is locked.
Medically Attended Adverse Events will be followed through the end of the study, a full 12 months following the last vaccination. After clinic visits end, follow-up for MAAEs will occur by monthly phone calls for two months.
For those participants who are unable to continue participation in the study, but who do not withdraw consent, an exit visit will be conducted.
5 Research labs may be performed at any visit where sufficient stored samples are available
6 Blood volumes for clinical lab assays may be adjusted as long as maximum daily blood volume is not exceeded.
7 Pregnancy tests can be conducted at any visit if clinically indicated.
8 Any clinically indicated safety labs can be obtained at investigator discretion

4.2. Discussion of Study Design

This study design is a randomized, double-blind study, with three study arms of equal allocation (1:1:1) to assess the safety, tolerability, and immunogenicity of ranging doses of candidate vaccine A244/B.63521 with ALFQ adjuvant.

4.3. Selection of Study Population

The study population will consist of 60 healthy adults not living with HIV randomized to receive active vaccine with varying levels of ALFQ adjuvant. All study participants must meet the eligibility criteria enumerated in the protocol (protocol version 1.0, sections 5.3.1, 5.3.2).

4.3.1. Sentinel Group

As this is a first-in human study, a sentinel group will first be enrolled to conform to the FDA suggestion in order to assess for safety and tolerability in a subset of participants prior to enrolling all 60 participants for the study. A total of six participants will comprise a sentinel group. Four participants will be randomized to the 200 mcg ALFQ group (Arm 1), one participant will be randomized to the 100 mcg ALFQ group (Arm 2), and one participant will be randomized to the 50 mcg ALFQ group (Arm 3), so the blinding is preserved. Safety data per diary card for seven days following vaccination number one will be reviewed by a PSRT. If it is determined to be necessary by the PSRT, the MHRP Safety Monitoring Committee (SMC) will be consulted to review unblinded data to provide a recommendation on continuing enrollment. No formal statistical analyses are planned for this review.

4.4. Statistical Considerations for the Study Design

4.4.1. Sample Size Considerations

The safety, reactogenicity, and tolerability of the vaccine regimens are the primary endpoints for this study. The table below demonstrates effect size with a power of 80%, calculated at a variety of sample sizes.

Table S3 depicts SAE or AESI rates and associated 95% confidence intervals in each potential grouping of interest given 0-5 participants with observed events. If we were to observe zero events within a single active study group (n=20), the upper limit of a 2-sided exact 95% CI would be 13.9% and true events above this could be ruled out at the α=0.025 level.

Table S4: Exact 95% Clopper-Pearson CI for the SAE Rate in Each Study Group

Number of Participants with Event	All Active Groups (n=60)	Any Active Group (n=20)
0	0% (0%, 4.9%)	0% (0%, 13.9%)
1	1.7% (<0.1%, 8.9%)	5.0% (0.1%, 24.9%)
2	3.3% (0.4%, 11.5%)	10.0% (1.2%, 31.7%)
3	5.0% (1.0%, 13.9%)	15.0% (3.2%, 37.9%)
4	6.7% (1.8%, 16.2%)	20.0% (5.7%, 43.7%)

Number of Participants with Event	All Active Groups (n=60)	Any Active Group (n=20
5	8.3% (2.8%, 18.4%)	25.0% (8.7%, 49.1%)

Table S4 presents the estimated power for the secondary outcome of antibody titer based on the published data from Pitisuttithum P et.al[1] . For the analysis of this experiment, we plan on observing a single measurement of peak antibody level per participant. Thus, the study is designed around a 1-way ANOVA model. Tukey's method will be used in the pairwise comparison test. In the analysis, we looked at the size of the error bars on the peak of the log antibody titer and based upon our visual inspection we assumed that the residual standard deviation is = 0.2. A sample size of approximately 20 participants per arm was selected to provide ≥80% power to detect the effect size of ≥ 0.202 between study arms. Statistical significance will be set at p≤0.05.

Table S5: Sample size per arm and minimum effect size with 80% power, assuming residual standard deviation of 0.2

	Part A: 3 Active arms	
n per arm	Minimum effect size	Power
15	0.236	0.803
16	0.228	0.804
17	0.220	0.801
18	0.214	0.803
19	0.208	0.803
20	**0.202**	**0.802**

4.4.2. Allocation of Participants to Study Arms (Randomization)

Randomization will be conducted using a modified permuted 1:1:1 randomization block scheme described in a separate randomization plan held by the protocol statistician. To maintain blinding of the sentinel group, four participants in the sentinel group will be randomized to the 200 mcg ALFQ group (Arm 1), and one participant each will be randomized to the 100 mcg ALFQ group (Arm 2) and50 mcg ALFQ group (Arm 3). Thereafter, when the study continues enrollment, randomizations will be administered in such a way as to achieve the eventual 1:1:1 overall balance. Once a randomization number has been assigned, it will not be re-assigned. If a participant withdraws from the study or discontinues vaccination before all slots have been filled, the vacated randomization slot will be filled with the next available participant while enrollment is open. The randomization list will be uploaded into REDCap by the DCAC data manager and will be accessed by the unblinded site pharmacist(s). The protocol statistician will generate a final listing of all randomizations with vaccinations dates upon completion of the study and after

the database has been locked (Listing 1). Additional randomization details are provided in the randomization plan.

4.5. Study Products

4.5.1. Study Products Administered

The study products administered include the glycoprotein A244, the recombinant HIV-1 Env protein B.63521, and the ALFQ adjuvant.

4.5.2. Identity of Investigational Product(s)

A244 consists of the gp120 envelope glycoprotein HIV-1 subtype CRF_01E A244 derived from the CM244 CRF_01AE. The A244 gp120 envelope has an 11 amino N-terminal deletion, similar to the A244 protein used in AIDSVAX B/E.

B.63521 gp120 is a recombinant HIV-1 Env protein containing an 11 amino acid truncation at the N-terminus of gp120 that enhances antigenicity and immunogenicity.

ALFQ (Army Liposomal Formulation) is a liposomal adjuvant containing a synthetic monophosphoryl lipid A (MPLA) with the addition of QS-21 (protocol version 1, section 6.1).

4.5.3. Selection and Timing of Dose for Each Participant

This is a three-dose study, with participants receiving the assigned study product of interest at Month 0 (Day 1), Month 1 (Day 29), and Month 2 (Day 57).

4.5.4. Blinding

The PI, study staff, and participants will be blinded as to group allocation as it pertains to the dose of ALFQ. The unblinded pharmacy team will maintain all of the accountability logs and other product related regulatory documents, such as completed preparatory sheets. These documents will not be accessible to the blinded team. The unblinded pharmacy team will be in charge of emergency access to unblinding. In addition, the delegated unblinded study staff/nurse preparing the vaccine syringes will not be involved in the clinical assessment of participants and will be instructed not to comment on the appearance of experimental agent to study staff. For all participants, the volume of injection will be consistent. Procedures used for drug packaging and distribution to maintain blinding are described in the pharmacy manual and the clinical manual of procedures. To prevent a blinded person from seeing the IP in the syringe, the unblinded nurses will use blinding tape to prevent blinded study staff from looking at the viscosity and color of the product while in the syringe.

In a non-emergency event when a unexpected serious adverse reaction occurs that is suspected (i.e. possible/probably related) to the treatment, the study PI or designee will send an unblinding request to rv575_unblinded@global-id.org, with ORA PSSO safety mailbox in copy, that includes the participant ID, reason for unblinding, and who should receive the unblinding information. The unblinded team at DCAC will provide the treatment assignment to the ORA PSSO representative within one business day of the request. Additional details are provided in the blinding management plan.

4.5.5. Prior and Concomitant Therapy

Concomitant medications will be recorded from 45 days prior to first vaccination until 10 months after the final vaccination. An initial review of past medical history will include a review of medications. Information pertaining to receipt of non-study vaccines, research agents, immunoglobulin preparations, immunosuppressive medication, antiretroviral drugs, and any blood products will be elicited at study visits and recorded in source documents.

To ensure appropriate medical follow-up for study participants, information regarding concomitant medications used in association with an AE will be collected and recorded in the source documents.

Otherwise, no other concomitant medication information will be collected.

4.5.6. Study Product Compliance

Any randomized participants who withdraw before receiving the study vaccination will be summarized in the participant disposition exhibits and reported in a listing. Those participants will be replaced by others determined to be eligible during screening visits.

4.6. Safety Variables

Safety will be assessed both by direct physical examination and by diary cards, which serve as memory tools for better identification of reactions. Participants will be asked to record their temperature and complete a diary card at home in the evening after the vaccination and each day for the next 14 days. During vaccination visits on Day 1, 29, and 57, participants will be assessed for symptoms of local (at the injection site) reactogenicity prior to administration and 30 minutes post-administration: pain/tenderness, itching, warmth, induration, redness, and for symptoms of systemic reactogenicity: fever, myalgia, arthralgia, headache, fatigue, chills, nausea, dizziness, and rash. After review of the diary card and discussion with the participant, the study staff will document any post-vaccination reaction(s) and all related information (severity, frequency, etc.) concerning such a reaction in the participant's clinic source documents. The frequency and incidence of specific post-vaccination reactions and any reaction will be used for analysis.

AEs and SAEs will be recorded at all visits along with timing and possible attribution to Investigational Product. Because this clinical trial involves an adjuvant, AESIs will also be assessed. As with solicited events, the frequency and incidence of specific post-vaccination reactions and any reaction will be used for analysis.

Safety laboratory analyses of complete blood count, liver function tests, and pregnancy test in females will also be performed according to the SOE. In addition to abnormal findings reported as AEs, changes from baseline will be computed.

Vital signs (body temperature, pulse, respiratory rate, and blood pressure) will be measured both pre- and at 30 minutes post-vaccination.

4.7. Immunogenicity Variables

Vaccine-induced immune responses will be assessed in study participants as detailed in the SOE.

Humoral responses will be assessed by HIV-specific binding antibody assays, HIV-specific neutralizing antibody assays, and non-neutralizing antibody function assays at baseline and days

1, 15, 29, 42, 57, 70, 168, and 336. Durability of IgG response will be assessed for each participant by estimating the decline in log10 IgG from peak to 6 and 12 months post final vaccination.

Measurement of CD4+ and CD8+ T-cell effector function will be evaluated through response by cytokines such as IFN-γ and IL2. CD4+ lymphoproliferation will be measured with the CFSE assay. Innate immunity (e.g., NK cells) will be assessed by quantifying ADCC and ADCP, determining NK cell phenotype using various flow cytometric panels, cytokine array assays to characterize the type of cytokines elicited by this vaccine regimen with or without adjuvants, by the assessment of gene expression by DNA microarray and related techniques, and other related assays. B cell responses will be characterized in blood and in diverse anatomical compartments (provided by sigmoid biopsies and lymph nodes biopsies) in a subset of participants. Host gene transcription profile and signature to vaccine antigens will be assessed by RNA sequencing and related techniques.

4.8. Other Variables

Age, gender, sex at birth, place of birth, level of education, occupation, race/ethnicity, and baseline medical history of participants will be recorded. Participant disposition variables will include the numbers of participants in each group and overall completing visits and study assessments.

5. GENERAL STATISTICAL CONSIDERATIONS

5.1. General Principles

All continuous variables, with appropriate transformations (e.g., log, square root, or arcsin) applied as necessary, will be summarized using the following descriptive statistics: n (non-missing sample size), mean, standard deviation, median, minimum, and maximum. The frequency and percentages (with the non-missing sample size as the denominator) of observed levels will be reported for all categorical measures. Exact Clopper-Pearson confidence intervals will be computed for binary variables, where applicable. For continuous variables, confidence intervals will be computed based on the t-distribution with n-1 degrees of freedom, where n denotes the number of observations. If the data are found to be non-normal, and the sample size is insufficient for application of the central limit theorem (which states that in sufficiently large samples, the sample mean is approximately normal), non-parametric bootstrapped confidence intervals may be calculated instead.

In general, all data listings will be sorted by treatment group and participant, and when appropriate by visit number within participant. All summary tables will be structured with a column for each treatment group in the order presented in the protocol and will be annotated with the total population size relevant to that table/treatment group, including any missing observations.

For hypothesis tests of continuous endpoints, an ANOVA will be preferred for global tests and t-tests will be preferred for pairwise tests. If histograms of the analysis data do not appear by visual inspection to be approximately normal, even after appropriate transformations, or if the sample sizes for comparisons groups are too small for the application of the central limit theorem, a Kruskal-Wallis test will be utilized instead of an ANOVA, and a Wilcoxon rank-sum test will be utilized instead of a t-test. For tests of categorical endpoints, a chi-square test or, if cell counts prohibit the use of a chi-square test, a Barnard's test or Fisher's exact test will be used as appropriate.

5.2. Timing of Analyses

The final study report will be completed when all primary objective data and secondary immunogenicity endpoint data are available. Additional exploratory immunogenicity analyses may be presented in separate reports and/or manuscripts, depending on data availability.

5.3. Analysis Populations

A tabular listing of all participants, visits, and observations excluded from the analysis populations will be provided in the final report. All data will be analysed based on intention-to-treat principles.

5.3.1. Safety Population

All participants who received at least one dose of the vaccination and for whom any post-dose data is available will be included in the safety population.

5.3.2. Immunogenicity Population

The Immunogenicity Population will include all data from randomized who have received at least one vaccination and have the necessary data available to analyze a given endpoint.

5.3.3. Per-Protocol Population

The Per-Protocol population will include participants in the immunogenicity population with additional exclusions as needed for sensitivity analyses. The per-protocol population will specifically exclude:

- Study visits and/or vaccinations occurring out-of-window as defined by the study protocol
- Study visits where a major protocol deviation occurred
- Study visits occurring after a participant becomes ineligible after randomization

5.4. Covariates and Subgroups

Demographics will be summarized using descriptive statistics, and there are no specific covariates of interest or explicit sub-group analyses planned.

5.5. Missing and Outlier Data

All attempts will be made to collect all data per protocol. Frequencies of missingness for primary outcomes will be calculated overall and within each study group. Less than 10% missing data that are missing completely at random will be considered ignorable missingness and complete case analysis will be utilized. No imputation will be performed for missing values. Data visualizations such as boxplots, histograms, or scatter plots will be used to identify outliers. Single outliers may be identified if values are outside of 1.5x the interquartile range depending on normality assumptions. Outliers will not be excluded from the primary analyses. Outliers identified during the analysis will be discussed in the analysis report.

Measurements that occur outside of study visit windows may be considered missing in sensitivity analyses. Table S5 provides an overview of each study visit along with the visit window upper and lower bounds, as specified in the study protocol.

Table S6. Analysis Time Windows

Visit	Visit day (visit window in days)	Lower bound (days)	Upper bound (days)
Screen	-45 to -3	-45	-3
Visit 1 (V1)	1	1	1
Visit 2 (Safety & Immun)	15 (±3)	12	18
Visit 3 (V2)	29 (±7)	22	36
Visit 4 (Safety & Immun)	43 (±3)	40	46
Visit 5 (V3)	57 (±7)	50	64

Visit	Visit day (visit window in days)	Lower bound (days)	Upper bound (days)
Visit 6 (Safety & Immun)	58-59	58	59
Visit 7 (Safety & Immun)	64 (±3)	61	67
Visit 8 (Safety & Immun)	71 (±3)	68	74
Visit 9 (Safety & Immun)	169 (±7)	162	176
Visit 10 /Exit 4 (Safety & Immun)	337 (±7)	330	344
Phone Follow-up 1 (MAAE)	365 (±7)	358	372
Phone Follow-up 2 (MAAE)	393 (±7)	386	400

Measurements that occur outside of the scheduled protocol-specified assessment time-windows will be flagged and assigned to the intended study visit. These measurements will be included in the primary analyses; sensitivity analyses will be conducted to determine whether the results differ when these measurements, which occurred outside of the scheduled protocol-specified assessment time-windows, are excluded. Although multiple measurements occurring within the same assessment window are not anticipated to be an issue, if such an event occurs the analytic approach will be determined based on the specific measure and accounting for clinical rationale and standard approaches in the literature. For example, the minimum value, maximum value, earliest value or latest value may be considered for sensitivity analyses.

5.6. Interim Analyses and Data Monitoring

No formal interim safety analyses are planned but may be performed if requested by the safety monitoring committee as outlined in protocol section 8.2.18.

Interim immunogenicity analyses will be conducted after all enrolled participants have reached at least week 10 (20.8% of follow-up accrual) and may be conducted at additional time points, if requested by the safety monitoring committee. The main goal of planned interim analyses through week 10 is to assess short-term effect of the candidate vaccine A244/B.63521 (300 µg each) with ALFQ adjuvant at the 50, 100, and 200 µg doses on cellular and humoral immune responses. This interim group analysis will not compromise the integrity of the trial in terms of the maintenance of the study blind, participant retention, or safety or immunogenicity endpoint assessments. All participant IDs will be replaced with alternate IDs by the unblinded study staff and only the minimum necessary summary level of information will be shared with blinded study staff. Results from planned interim immunogenicity analyses will be used to inform the design of a subsequent clinical trial.

In cases where specific immunogenicity analysis replicates end-of-study endpoints, to minimize the risk of type-I error, an alpha-spending function, such as O'Brien-Fleming boundary will be

used to monitor primary and secondary endpoints using an overall, two-sided alpha of 0.05, with the overall goal being to maximize the amount of alpha reserved for the final study analysis.

5.6.1. Humoral Immune Response Interim Analysis

The primary analytical question of interest in the interim analysis is whether significant differences in humoral immune response are observed between dosing regimens. Immune response variables will be determined by the WRAIR lab scientists dependent on sample quality and availability but, in general, will be reflective of those detailed in the primary endpoints (see protocol) and in Section 8 of the SAP. The three dosing groups will be assessed across four visits: weeks 0, 2, 6, and 10. Depending on the statistical technique, the null hypothesis will be that the means or medians do not differ by dosing group.

Analytical Approach for Immunogenicity Response

To assess differences in humoral immunogenicity response across the three dose groups, linear mixed effects models will be used. The immunogenicity response will be the outcome variable; if necessary, the outcome will be appropriately transformed for inferential analyses and transformed back to the original scale for reporting. The model will include time in days since first injection as a continuous variable, dose group, and an interaction effect between time and dose group. An appropriate method of accounting for covariance will be identified—a likely approach will be to have random intercepts and a random slope for time and an unstructured covariance pattern. A statistically significant interaction effect will indicate that there is a significant difference between groups in the trajectory of the response over time. If a significant interaction effect is observed, pairwise comparisons will be made between groups to determine which group differences explain the significant effect. This approach will not include comparisons between groups at any specific time points; this method precludes having to use an alpha-spending function as these analyses will be qualitatively distinct from those employed in the final analyses.

Area under the Response Curve

Mean area under the curve (AUC) can be used to represent the time and population averaged immune response from week 0 to week 10. Time will be measured in days since first injection and reflect the date of sample collection (rather than visit number). Immunogen response distribution will be assessed for each visit for overall homogeneity of response pattern independently for each group. Time-normalized values will be calculated for AUC (denoted as nAUC) to account for differences in follow-up time due to early drop-out or missing data. Individual nAUC may be calculated via the trapezoidal method as:

$$\frac{1}{T} \quad Y \quad \frac{\quad}{T} \quad \frac{\quad}{\tilde{2}\ 1} \quad +$$

Where is the normalized AUC for an individual; T_i=total observation time for individual i; $Y_{i,j}$=humoral response of individual i at time j, m_i=number of timepoints for individual i, $t_{i,j}$=time in days for individual i at time j.

If the data reflects apparent inflection points, a cubic spline function may be used to estimate the functional form of the response data, and the nAUC may be derived using the definite integral of

the response curve from v0 to v10 to account for the data's inflection tendencies. Estimation of nAUC may also be derived through applications of a linear mixed effect model following methods outlined in Alexandre (2021) for group level effects, with or without the inclusion of additional covariates.

ANOVA will be used to assess differences in AUC between groups. Normality assumptions for immunogenicity marker AUC will be assessed. If strong departures from normality are observed data will be transformed to achieve normality; log transformations will be prioritized but other transformations will be explored as appropriate for optimal fit. In the case where normality transformations perform poorly, a Kruskal-Wallis analysis will be used incorporating appropriate adjustments for equivalence testing.

5.6.2. Cellular Immune Response Interim Analysis

Cellular immune responses mediated by T lymphocytes will also be assessed in the interim analysis. Cellular immune response variables will be determined by the WRAIR lab scientists dependent on sample quality and availability but, in general, will be reflective of those detailed in the primary endpoints (see protocol) and in Section 8 of the SAP. The three dosing groups will be assessed across four visits: weeks 0, 2, 6, and 10. Depending on the statistical technique, the null hypothesis will be that the means or medians do not differ by dosing group.

Analytical Approach for Cellular Immunogenicity Response
The overall magnitude and quality of the antigen-specific T cell response will be assessed through detection of cytokine expression measured by flow cytometry. Measures at pre-vaccination and 2 weeks after each vaccination (weeks 2, 6, 10) will be assessed from all individuals divided by vaccination group. Levels of antigen-specific T cells will be compared between groups at both timepoints for Total responses (Boolean gating of flow cytometry data will be used to assess) and individual cytokines (IL-21, IL-2, TNF-a, IL-4, IFN-γ, CD154). Polyfunctional scores and Heatmap of posterior probabilities via COMPASS analysis will be assessed using R software.

5.7. Multiple Comparisons/Multiplicity

All hypothesis tests will be performed as two-sided at the 5%-level, including planned immunogenicity analysis. Due to the limited sample size and the early phase of the study, adjustment for multiplicity across endpoints will not occur. For individual endpoints that involve a multitude of hypothesis testing (e.g., gene expression), the analysis will be adjusted for multiplicity using the Bonferroni-Holm step-down procedure [3]. Results will be considered on the strength of evidence. P-values ≤ 0.05 will be considered statistically significant evidence of a difference or association.

6. STUDY PARTICIPANTS

6.1. Disposition of Participants

The number of participants who enroll in the trial, and the number and percentage of participants who complete each assessment, will be presented by vaccination group. A flowchart showing the disposition of study participants, adapted from the Consort Statement, will be included, including the number of participants enrolled, withdrawn, lost to follow-up, and analysed by treatment group. The percentage of participants who withdraw from the trial or discontinue the study drug, and reasons for withdrawal or discontinuation, will be summarized by group and given in a listing.

6.2. Demographic and Other Baseline Characteristics

Participant demographics and baseline characteristics, listed in section 4.9, will be summarized by vaccination group, using the statistics described in Section 5.1.

6.3. Prior and Concurrent Medical Conditions

Prior and concurrent medical conditions, collected as part of medical history assessments, will be summarized by vaccination group. Any medical conditions reported subsequent to enrollment that meets study exclusion criteria will be presented in a listing.

6.4. Prior and Concomitant Medications

Any concomitant medications reported subsequent to enrollment that meet study exclusion criteria will be presented in a listing.

6.5. Measurements of Study Product Compliance

A listing presenting each participant's vaccination group and product received will be presented. Any participants who receive the wrong study vaccination and those who are randomized but not vaccinated will also be presented in a line listing.

6.6. Protocol Deviations

All major protocol deviations will be summarized by vaccination group and presented in a listing. A summary of protocol deviations by vaccination group will also be presented in a table.

7. SAFETY EVALUATION

All summaries and analyses of safety data will be presented for the Safety Population. Safety summaries for the occurrence and severity of SAEs, AEs, AESIs, and unsolicited AES will be presented by vaccination group. The denominator for the percentages may be based on the number of non-missing observations for an assessment or based on the number of participants in a population. This will be described for each exhibit.

7.1.1. Solicited Reactogenicity Events

The occurrence of local and systemic reactogenicity symptoms will be listed and summarized for each vaccination group as the proportions of patients experiencing safety events along with 95% exact Clopper-Pearson CI for the underlying probability of the event. These will be described overall. Differences in the proportion of patients with safety outcomes in each group will be assessed using either the Fisher exact test, or Pearson chi-square test, as appropriate.

The likelihood of reporting solicited local and systemic AEs will be compared across groups via exact binomial tests. If computational challenges arise, Chi-squared tests may be used instead.

7.1.2. Unsolicited Adverse Events

The occurrence of unsolicited AEs, SAEs, AESIs, and new-onset medical conditions will be listed by body system and preferred term for each vaccination arm. Unsolicited AEs will be assessed with the number and proportion of participants experiencing each safety event at any point during the reactogenicity period, along with the associated exact 95% CI. These will be described overall. A participant will be counted only once per PT per reporting period. Unsolicited AEs will also be tabulated by investigator-determined relationship to investigational product and severity for each vaccination group.

7.1.3. Clinical Laboratory Data

Clinical laboratory values, including change from baseline, will be summarized by vaccination group. The values will be graded according to DAIDS Table for Grading the Severity of Adult and Pediatric Adverse Events (DAIDS AE Grading Table), Version 2.1 dated July 2017 that is found on the website https://rsc.niaid.nih.gov/clinical-research-sites/daids-adverse-event-grading-tables, and, reported as AEs. All other events will be graded according to the scale in Table S7. The case report form for adverse events will reflect only the highest severity for continuous days an event occurred.

Table S7: DAIDS Adverse Event Grading Scale

Estimating Severity Grade for Parameters Not Identified in the Grading Table

The functional table below should be used to grade the severity of an AE that is not specifically identified in the grading table. In addition, all deaths related to an AE are to be classified as grade 5.

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
Clinical adverse event **NOT** identified elsewhere in the grading table	Mild symptoms causing no or minimal interference with usual social & functional activities with intervention not indicated	Moderate symptoms causing greater than minimal interference with usual social & functional activities with intervention indicated	Severe symptoms causing inability to perform usual social & functional activities with intervention or hospitalization indicated	Potentially life-threatening symptoms causing inability to perform basic self-care functions with intervention indicated to prevent permanent impairment, persistent disability, or death

Clinical labs will be presented by visit, grade, and vaccination group. Additionally, descriptive summary statistics (mean, SD, median, minimum, maximum) will be presented for each clinical lab. Change from screening summaries will be done for each follow-up visit. For change-from-screening summaries, participants with an undefined change from screening, due to missing data, will be excluded.

Clinical laboratory values will be presented in listings as well.

8. IMMUNOGENICITY EVALUATION

8.1. Primary Immunogenicity Analyses

All summaries and analysis of immunogenicity data will be presented for the Immunogenicity Population. Humoral and cellular immunogenicity will be assessed primarily through descriptive statistics. Primary immunogenicity endpoints are IgG binding antibodies to g120 and antigen-specific CD4 and CD8 T cell responses.

The magnitude of plasma IgG (antigen-specific) responses will be measured by the endpoint titer at study weeks 0, 2, 4, 6, 8, 10, 24 and 48. Means or Geometric mean titers (GMTs) will be calculated for each group as appropriate based on the distribution, at each visit, and the per-participant peak response across all visits. Additionally, 95% confidence intervals (CI) will be computed using bootstrapping, or alternative methods. Results will be presented as exponentiated GMT and 95% CI. The distributions of GMTs will be compared across all vaccination groups via ANOVA (null hypothesis that the medians across all groups do not differ) at the 5%-statistical significance level, or using the Kruskal-Wallis test if ANOVA assumptions are violated; in the event Kruskal-Wallis testing is implemented the shape and scale uniformity across groups will be assessed for validity and results interpretation. Pairwise comparisons of GMT responses between dose groups at follow-up time points will be evaluated using a two-sided Mann-Whitney U test, with a Bonferroni-Holm step-down for adjusting p-values for multiple comparisons. Where necessary and appropriate, False Discovery Rates (FDR) will be calculated and reported using the Benjamini-Hochberg (BH) method.

The number and percentage of positive IgG responses to binding targets will be presented by vaccination group and visit based on input from the laboratory team. Differences between vaccinations groups will be evaluated using Fisher's exact test, or chi-squared tests.

Durability of IgG response will be assessed for each participant by estimating the decline in $\log_{10}$ IgG from peak to 6 and 12 months (weeks 24 and 48 respectively) post final vaccination.

Total immune responses over time will be assessed by the positive incremental area under the curve (AUC). An AUC curve will be constructed with raw, unlogged endpoint titers on the y-axis and visit week on the x-axis (week 0 to week 48). AUC will be logged after calculation from raw values. For comparability between participants with different numbers of samples (visits) available, these values will be scaled (normalized) by the week of the last available sample. If no sample is available for visits other than the first and last, no explicit imputation will be done, and the AUC will be calculated based on available samples. Log AUC will be analysed similarly to the visit-by-visit titers and presented as mean AUC and 95% CI.

The magnitude of CD4+ and CD8+ T-cell effector function and polyfunctionality will be evaluated through response by cytokines such as IFN-ɣ and IL2. The frequencies of CD4 or CD8 T cells responding to each test antigen with IFN-ɣ and/or IL2 will be calculated using the following categories: all IFNɣ+, all Il2+, IFNɣ and IL2+, IFNɣ or IL2+. Descriptive statistics will evaluate the distribution of CD4+ and CD8+ responses and polyfunctionality scores by vaccination group and visit. Differences in CD4+ and CD8+ responses between vaccination groups at each visit will be evaluated using Kruskal-Wallis tests, and pair-wise comparisons of

particular visits will be performed with two-sided Mann-Whitney U test, with a Bonferroni-Holm step-down for adjusting p-values for multiple comparisons.

8.2. Additional Immunogenicity Analyses

Further immunogenicity analyses (see Section 3.2.3 for planned sets of assays) will be considered exploratory for testing purposes (see Section 5.8 for how significance and multiplicity will be handled), but they will otherwise follow a similar process as the primary immunogenicity analysis. Secondary immunogenicity endpoints will entail primarily descriptive analyses. In general, continuous outcomes will be assessed using summary statistics such as the mean, median, standard deviation and IQR. Binary responses will be described using frequencies and percentages. Changes from baseline may be assessed within groups using paired t-tests (or Wilcoxon signed rank as appropriate) or McNemar's test for binary outcomes. For assays that are not conducted via titer experiments, the analysis values will be handled as log GMTs, after appropriate transformation of the data to be approximately normal when necessary; boxplots will show the assay values for each group, by visit.

8.3. Immunogenicity Sensitivity Analyses

Primary immunogenicity analyses conducted in the immunogenicity population may be repeated in the per-protocol population if per-protocol conditions are met. Results of primary analyses will be considered to be robust if effects are similar.

Examples of per-protocol sensitivity analyses include excluding study visits occurring out-of window, or where a major protocol deviation occurred. Bayes factors may be calculated and assessed for sensitivity analyses assessment.

If imbalance in participant demographic or clinical factors is detected between vaccination groups at the time of study enrolment, additional sensitivity analyses may adjust for imbalances in multivariable adjusted models.

Lastly, the presence of outliers will be evaluated using z-scores or boxplots for distributions, and if detected, sensitivity analyses may be performed excluding observations flagged as outliers.

9. REPORTING CONVENTIONS

P-values ≥0.001 and ≤0.999 will be reported to 3 decimal places; p-values less than 0.001 will be reported as "<0.001" and p-values greater than 0.999 will be reported as >0.999. The mean, standard deviation, and other statistics will be reported to 1 decimal place greater than the original data. The minimum and maximum will use the same number of decimal places as the original data. Proportions will be presented as 2 decimal places; values greater than zero but less than 0.01 will be presented as "<0.01" and values greater than 0.99 and less than one will be presented as ">0.99". Percentages will be reported to the nearest whole number; values greater than zero but < 1% will be presented as "<1"; values greater than 99% but less than 100% will be reported as >99%. Estimated parameters, not on the same scale as raw observations (e.g., regression coefficients) will be reported to 3 significant figures unless otherwise specified in the output shells.

10. TECHNICAL DETAILS

Clinical statistical analysis will be performed using Statistical Analysis Software (SAS®) version 9.4 (Cary, North Carolina, USA) and/or R version 4.0 or above. Other software may be used for processing of assays, and further details will be presented in the final report if they are available.

11. SUMMARY OF CHANGES IN THE CONDUCT OF THE STUDY OR PLANNED ANALYSES

Any substantial changes to the analysis plan in the protocol prior to final database lock will be presented here. The SAP will be reviewed and updated prior to unblinding.

11.1 Interim Analysis Plan Alterations—December 2023

Additional content was added to section 5.6 to detail the expected analysis for the Interim Immunogenicity analysis. Blinded group analysis will be conducted for various immunologic parameters up through week 10 once all participants have been enrolled. Analysis of variance (ANOVA), non-parametric group tests, and group AUC will be used to assess the group short-term response. Adjustments for multiple comparisons, false discovery (FDR), and effect size (i.e. Bayes factors) will be used as appropriate.

12. REFERENCES

1. Pitisuttithum P, Nitayaphan S, Chariyalertsak S, et al. Late boosting of the RV144 regimen with AIDSVAX B/E and ALVAC-HIV in HIV-uninfected Thai volunteers: a double-blind, randomised controlled trial. Lancet HIV. 2020 Apr;7(4):e238-e248. doi: 10.1016/S2352-3018(19)30406-0. Epub 2020 Feb 6.
2. Marie Alexandre, Mélanie Prague, Rodolphe Thiébaut. Between group comparison of AUC in clinical trials with censored follow-up: Application to HIV therapeutic vaccines. Statistical Methods in Medical Research, 2021, 10.1177/09622802211023963. hal-03241637v2

APPENDIX 1: ADDITIONAL TABLES AND FIGURES

Table 1: Participant Disposition by Vaccination Group

Participant Disposition	200 µg ALFQ (N=X)		100 µg ALFQ (N=X)		50 µg ALFQ (N=X)		All Participants (N=X)	
	n	%	n	%	n	%	n	%
Screened	--	--	--	--	--	--	--	--
Enrolled/Randomized	x	100	x	100	x	100	x	100
Received Study Vaccination Dose 1 (Visit 1, Day 1)	x	xx	x	xx	x	xx	x	xx
Completed Visit 2 (Day 15)	x	xx	x	xx	x	xx	x	xx
Received Study Vaccination Dose 2 (Visit 3, Day 29)	x	xx	x	xx	x	xx	x	xx
Completed Visit 4 (Day 43)	x	xx	x	xx	x	xx	x	xx
Received Study Vaccination Dose 3 (Visit 5, Day 57)	x	xx	x	xx	x	xx	x	xx
Completed Visit 6 (Day 58-9)	x	xx	x	xx	x	xx	x	xx
Completed Visit 7 (Day 64)	x	xx	x	xx	x	xx	x	xx
Completed Visit 8 (Day 71)	x	xx	x	xx	x	xx	x	xx
Completed Visit 9 (Day 169)	x	xx	x	xx	x	xx	x	xx
Completed Visit 10/Exit (Day 337)	x	xx	x	xx	x	xx	x	xx
Phone Follow-up #1 (Day 365)	x	xx	x	xx	x	xx	x	xx
Phone Follow-up #2 (Day 393)	x	xx	x	xx	x	xx	x	xx
Note: N = Number of randomized participants								

Table 2: Summary of Demographic and Baseline Characteristics by Vaccination Group

Variable	Characteristic	200 µg ALFQ (N=X)		100 µg ALFQ (N=X)		50 µg ALFQ (N=X)		All Participants (N=X)		p-value
		n	%	n	%	n	%	n	%	
Age	18-29	x	xx	x	xx	x	xx	x	xx	
	30-39									
	40-49									
	50+									
Age, years	Mean									
	Standard Deviation									
	Median									
	Q1 (25th percentile)									
	Q2 (75th Percentile)									
Gender	Male									
	Female									
	Transgender									
Sex at birth	Male									
	Female									
	Undifferentiated									
Level of Education										
	Primary education									
	Secondary education									
	Undergraduate degree									
	Graduate									
	Other									
Race	White									
	Black or African American									
	Asian									
	American Indian/Alaskan Native									
	Native Hawaiian or Other Pacific Islander									
	Multiracial									
	Other									
Ethnicity	Non-Hispanic/Latino									

Variable	Characteristic	200 µg ALFQ (N=X)		100 µg ALFQ (N=X)		50 µg ALFQ (N=X)		All Participants (N=X)		p-value
		n	%	n	%	n	%	n	%	
	Hispanic/Latino									

Table 3: Summary of Participants with Pre-Existing Medical Conditions by Body System and Vaccination Group

	200 µg ALFQ (N=X)		100 µg ALFQ (N=X)		50 µg ALFQ (N=X)		All Participants (N=X)	
Body System	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**
General	x	x.x	x	x.x	x	x.x	x	x.x
HEENT	x	x.x	x	x.x	x	x.x	x	x.x
Cardiovascular								
Respiratory								
Gastrointestinal/Digestive								
Genitourinary								
Musculoskeletal								
Neurologic								
Endocrine								
Psychiatric								
Hematologic/Lymphatic								
Metabolic/Nutritional								
Allergy/Drug Sensitivity								
Other								

Table 4: Distribution of Protocol Deviations by Deviation Category and Vaccination Group

Deviation Category	200 µg ALFQ (N=X)		100 µg ALFQ (N=X)		50 µg ALFQ (N=X)		All Participants (N=X)	
	No. of Part.	No. of Dev.	No. of Part.	No. of Dev.	No. of Part.	No. of Dev.	No. of Part.	No. of Dev.
Enrollment	x	x	x	x	x	x	x	x
Informed consent	x	x	x	x	x	x	x	x
Visit schedule adherence	x	x	x	x	x	x	x	x
Protocol procedure	x	x	x	x	x	x	x	x
Adverse event not reported	x	x	x	x	x	x	x	x
Infusion schedule	x	x	x	x	x	x	x	x
Other	x	x	x	x	x	x	x	x

Note: Example categories and deviation types given

SAFETY SUMMARY

Table 5: Overall Summary of Adverse Events

	200 µg ALFQ (N=X)		100 µg ALFQ (N=X)		50 µg ALFQ (N=X)		All Participants (N=X)	
Participants[a] with	n	%	n	%	n	%	n	%
At least one local solicited reactogenicity event	x	x.x	x	x.x	x	x.x	x	x.x
Mild (Grade 1)								
Moderate (Grade 2)								
Severe (Grade 3)								
Potentially Life Threatening (Grade 4)								
At least one systemic solicited reactogenicity event	x	x.x	x	x.x	x	x.x	x	x.x
Mild (Grade 1)								
Moderate (Grade 2)								
Severe (Grade 3)								
Potentially Life Threatening (Grade 4)								
At least one unsolicited adverse event	x	x.x	x	x.x	x	x.x	x	x.x
At least one related unsolicited adverse event	x	x.x	x	x.x	x	x.x	x	x.x
Mild (Grade 1)	x	x.x	x	x.x	x	x.x	x	x.x
Moderate (Grade 2)	x	x.x	x	x.x	x	x.x	x	x.x
Severe (Grade 3)	x	x.x	x	x.x	x	x.x	x	x.x
Potentially Life Threatening (Grade 4)								
Fatal (Grade 5)								
At least one severe (Grade 3) unsolicited adverse event	x	x.x	x	x.x	x	x.x	x	x.x
Related	x	x.x	x	x.x	x	x.x	x	x.x
Unrelated	x	x.x	x	x.x	x	x.x	x	x.x
At least one adverse event of special interest	x	x.x	x	x.x	x	x.x	x	x.x
At least one serious adverse event[b]	x	x.x	x	x.x	x	x.x	x	x.x
At least one related, serious adverse event	x	x.x	x	x.x	x	x.x	x	x.x
At least one adverse event leading to early termination	x	x.x	x	x.x	x	x.x	x	x.x

Table 6: Adverse Events Occurring in 5% of Participants in Any Vaccination Group by System Organ Class and Preferred Term, and Vaccination Group

System Organ Class	Preferred Term	200 µg ALFQ (N=X)			100 µg ALFQ (N=X)			50 µg ALFQ (N=X)			All Participants (N=X)		
		n	%	Events	n	%	Events	n	%	Events	n	%	Events
Serious Adverse Events													
All	All	x	x.x	x	x	x.x	x	x	x.x	x	x	x.x	x
SOC 1	PT1	x	x.x	x	x	x.x	x	x	x.x	x	x	x.x	x
Etc.	Etc.												
Other (Non-Serious) Adverse Events													
All	All	x	x.x	x	x	x.x	x	x	x.x	x	x	x.x	x
SOC 1	PT1	x	x.x	x	x	x.x	x	x	x.x	x	x	x.x	x
Etc.	Etc												
Solicited Adverse Events													
All	All	x	x.x	x	x	x.x	x	x	x.x	x	x	x.x	x
SOC 1	PT1	x	x.x	x	x	x.x	x	x	x.x	x	x	x.x	x
Etc.	Etc												

Note: N = Number of participants in the Safety Population; n = Number of participants reporting event; Events = Total frequency of events reported.

Table 7: Collapsed Comparisons of the Proportion of Participants Experiencing Adverse Events by Event Type

Event Type	200 µg ALFQ (N=X) n (%)	100 µg ALFQ (N=X) n (%)	50 µg ALFQ (N=X) n (%)	p-value 0.xx
Solicited Adverse Events				
Any Solicited Symptom	x (x.x)	x (x.x)	0.xx	0.xx
Mild (grade 1)[b]	x (x.x)	x (x.x)	0.xx	0.xx
Moderate (Grade 2)[b]	x (x.x)	x (x.x)	0.xx	0.xx
Severe (Grade 3)[b]	x (x.x)	x (x.x)	0.xx	0.xx
Potentially Life Threatening (Grade 4)[b]	x (x.x)	x (x.x)	0.xx	0.xx
Any Systemic Symptom	x (x.x)	x (x.x)	0.xx	0.xx
Mild (grade 1)[b]	x (x.x)	x (x.x)	0.xx	0.xx
Moderate (Grade 2)[b]	x (x.x)	x (x.x)	0.xx	0.xx
Severe (Grade 3)[b]	x (x.x)	x (x.x)	0.xx	0.xx
Potentially Life Threatening (Grade 4)[b]	x (x.x)	x (x.x)	0.xx	0.xx
Any Local Symptom	x (x.x)	x (x.x)	0.xx	0.xx
Mild (grade 1)[b]	x (x.x)	x (x.x)	0.xx	0.xx
Moderate (Grade 2)[b]	x (x.x)	x (x.x)	0.xx	0.xx
Severe (Grade 3)[b]	x (x.x)	x (x.x)	0.xx	0.xx
Potentially Life Threatening (Grade 4)[b]	x (x.x)	x (x.x)	0.xx	0.xx
Unsolicited Adverse Events				
Any Serious Adverse Event	x (x.x)	x (x.x)	0.xx	0.xx
Any Adverse Event of Special Interest	x (x.x)	x (x.x)	0.xx	0.xx
Any Related, Unexpected Adverse Event	x (x.x)	x (x.x)	0.xx	0.xx
Any Unexpected Adverse Event Within 28 Days of Study Vaccination	x (x.x)	x (x.x)	0.xx	0.xx

Note: N = Number of participants in the Safety Population; n = Number of participants reporting each symptom [a]Exact binomial tests used

[b]Maximum severity reported

SOLICITED ADVERSE EVENTS

Table 8: Number and Percentage of Participants Experiencing Solicited Reactogenicity Events with 95% Confidence Intervals, by Event and Vaccination Group

	200 µg ALFQ (N=X)			100 µg ALFQ (N=X)			50 µg ALFQ (N=X)			All Participants (N = X)		
Event	**n**	**%**	**95% CI**	**n**	**%**	**95% CI**	**n**	**%**	**95% CI**	**n**	**%**	**95% CI**
Any Reactogenicity	x	x.x	x.x, x.x	x	x.x	x.x, x.x	x	x.x	x.x, x.x	x	x.x	x.x, x.x
Any Systemic Reactogenicity	x	x.x	x.x, x.x	x	x.x	x.x, x.x	x	x.x	x.x, x.x	x	x.x	x.x, x.x
[Systemic Event 1]	x	x.x	x.x, x.x	x	x.x	x.x, x.x	x	x.x	x.x, x.x	x	x.x	x.x, x.x
[Systemic Event 2]	x	x.x	x.x, x.x	x	x.x	x.x, x.x	x	x.x	x.x, x.x	x	x.x	x.x, x.x
Any Local Reactogenicity	x	x.x	x.x, x.x	x	x.x	x.x, x.x	x	x.x	x.x, x.x	x	x.x	x.x, x.x
[Local Event 1]	x	x.x	x.x, x.x	x	x.x	x.x, x.x	x	x.x	x.x, x.x	x	x.x	x.x, x.x
[Local Event 2]	x	x.x	x.x, x.x	x	x.x	x.x, x.x	x	x.x	x.x, x.x	x	x.x	x.x, x.x

Note: N = Number of participants in the Safety Population

Table 9: Number and Percentage of Participants Experiencing Solicited Reactogenicity Events by Event, Maximum Severity, and Vaccination Group

X.X

Event	Severity	200 µg ALFQ (N=X)		100 µg ALFQ (N=X)		50 µg ALFQ (N=X)	
		n	%	n	%	n	%

Any Reactogenicity	None	x	x.x	x	x.x	x	x.x
	Mild	x	x.x	x	x.x	x	x.x
	Moderate	x	x.x	x	x.x	x	x.x
	Severe	x	x.x	x	x.x	x	x.x
	Potentially Life Threatening						
Systemic Reactogenicity							
Any Systemic Reactogenicity	None	x	x.x	x	x.x	x	x.x
	Mild	x	x.x	x	x.x	x	x.x
	Moderate	x	x.x	x	x.x	x	x.x
	Severe	x	x.x	x	x.x	x	x.x
	Potentially Life Threatening						
[Systemic Event 1]	None	x	x.x	x	x.x	x	x.x
	Mild	x	x.x	x	x.x	x	x.x
	Moderate	x	x.x	x	x.x	x	x.x
	Severe	x	x.x	x	x.x	x	x.x
	Potentially Life Threatening						
[Systemic Event 2]	None	x	x.x	x	x.x	x	x.x
	Mild	x	x.x	x	x.x	x	x.x
	Moderate	x	x.x	x	x.x	x	x.x
	Severe	x	x.x	x	x.x	x	x.x
	Potentially Life Threatening						
Local Reactogenicity							
Any Local Reactogenicity	None	x	x.x	x	x.x	x	x.x
	Mild	x	x.x	x	x.x	x	x.x
	Moderate	x	x.x	x	x.x	x	x.x
	Severe	x	x.x	x	x.x	x	x.x
	Potentially Life Threatening						
[Local Event 1]	None	x	x.x	x	x.x	x	

Event	Severity	200 µg ALFQ (N=X)		100 µg ALFQ (N=X)		50 µg ALFQ (N=X)	
		n	%	n	%	n	%
	Mild	x	x.x	x	x.x	x	x.x
	Moderate	x	x.x	x	x.x	x	x.x
	Severe	x	x.x	x	x.x	x	x.x
	Potentially Life Threatening						
[Local Event 2]	None	x	x.x	x	x.x	x	x.x
	Mild	x	x.x	x	x.x	x	x.x
	Moderate	x	x.x	x	x.x	x	x.x
	Severe	x	x.x	x	x.x	x	x.x
	Potentially Life Threatening						

Note: N = Number of participants in the Safety Population who received the specified dose; n = Number of participants reporting each event; severity is the maximum severity reported post dosing for each participant for each day.

UNSOLICITED ADVERSE EVENTS

Table 10: Summary of Unsolicited Adverse Events by System Organ Class and Preferred Term, and Vaccination Group

System Organ Class	Preferred Term	200 µg ALFQ (N=X)				100 µg ALFQ (N=X)				50 µg ALFQ (N=X)			
		n	%	95% CI	Events	n	%	95% CI	Events	n	%	95% CI	Events
Serious Adverse Events													
Any	Any	x	x.x	x.x, x.x	x	x	x.x	x.x, x.x	x	x	x.x	xx, xx	x
[SOC 1]	Any	x	x.x	x.x, x.x	x	x	x.x	x.x, x.x	x	x	x.x	xx, xx	x
	[PT 1]	x	x.x	x.x, x.x	x	x	x.x	x.x, x.x	x	x	x.x	xx, xx	x
	[PT 2]	x	x.x	x.x, x.x	x	x	x.x	x.x, x.x	x	x	x.x	xx, xx	x
[SOC 2]	Any	x	x.x	x.x, x.x	x	x	x.x	x.x, x.x	x	x	x.x	xx, xx	x
	[PT 1]	x	x.x	x.x, x.x	x	x	x.x	x.x, x.x	x	x	x.x	xx, xx	x
	[PT 2]	x	x.x	x.x, x.x	x	x	x.x	x.x, x.x	x	x	x.x	xx, xx	x
Adverse Events of Special Interest													
Any	Any	x	x.x	x.x, x.x	x	x	x.x	x.x, x.x	x	x	x.x	xx, xx	x
[SOC 1]	Any	x	x.x	x.x, x.x	x	x	x.x	x.x, x.x	x	x	x.x	xx, xx	x
	[PT 1]	x	x.x	x.x, x.x	x	x	x.x	x.x, x.x	x	x	x.x	xx, xx	x
	[PT 2]	x	x.x	x.x, x.x	x	x	x.x	x.x, x.x	x	x	x.x	xx, xx	x
[SOC 2]	Any	x	x.x	x.x, x.x	x	x	x.x	x.x, x.x	x	x	x.x	xx, xx	x
	[PT 1]	x	x.x	x.x, x.x	x	x	x.x	x.x, x.x	x	x	x.x	xx, xx	x
	[PT 2]	x	x.x	x.x, x.x	x	x	x.x	x.x, x.x	x	x	x.x	xx, xx	x
Other Non-Serious Unsolicited Adverse Events													
Any	Any	x	x.x	x.x, x.x	x	x	x.x	x.x, x.x	x	x	x.x	xx, xx	x
[SOC 1]	Any	x	x.x	x.x, x.x	x	x	x.x	x.x, x.x	x	x	x.x	xx, xx	x
	[PT 1]	x	x.x	x.x, x.x	x	x	x.x	x.x, x.x	x	x	x.x	xx, xx	x

System Organ Class	Preferred Term	200 µg ALFQ (N=X)				100 µg ALFQ (N=X)				50 µg ALFQ (N=X)			
		n	%	95% CI	Events	n	%	95% CI	Events	n	%	95% CI	Events
	[PT 2]	x	x.x	x.x, x.x	x	x	x.x	x.x, x.x	x	x	x.x	xx, xx	x
[SOC 2]	Any PT	x	x.x	x.x, x.x	x	x	x.x	x.x, x.x	x	x	x.x	xx, xx	x
	[PT 1]	x	x.x	x.x, x.x	x	x	x.x	x.x, x.x	x	x	x.x	xx, xx	x
	[PT 2]	x	x.x	x.x, x.x	x	x	x.x	x.x, x.x	x	x	x.x	xx, xx	x

Note: N = Number of participants in the Safety Population; n = Number of participants reporting each SOC/PT. A participant is only counted once per PT.

Table 11: Number and Percentage of Participants Experiencing Unsolicited Adverse Events by System Organ Class and Preferred Term, Maximum Severity, Relationship, and Vaccination Group

System Organ Class	Preferred Term	Any Incidence		Severity[1]								Relationship to Treatment[2]			
				Mild		Moderate		Severe		Potentially Life Threatening		Not Related		Related	
		n	%	n	%	n	%	n	%	n	%	n	%	n	%
	200 µg ALFQ (N=X)														
Any	Any PT	x	xx	x	xx	x	xx	x	xx	x	xx	x	xx	x	xx
[SOC 1]	Any PT														
	[PT 1]														
	[PT 2]														
[SOC 2]	Any PT														
	[PT 1]														
	[PT 2]														
	100 µg ALFQ (N=X)														
Any	Any PT	x	xx	x	xx	x	xx	x	xx	x	xx	x	xx	x	xx
[SOC 1]	Any PT														
	[PT 1]														
	[PT 2]														
[SOC 2]	Any PT														
	[PT 1]														
	[PT 2]														
	50 µg ALFQ (N=X)														
Any	Any PT	x	xx	x	xx	x	xx	x	xx	x	xx	x	xx	x	xx
[SOC 1]	Any PT														
	[PT 1]														
	[PT 2]														
[SOC 2]	Any PT														
	[PT 1]														
	[PT 2]														
	Note: N = Number of participants in the Safety Population. [1] For severity, a participant is counted once per preferred term and is summarized according to the highest reported severity. [2] For relationship, a participant is only counted once per preferred term and is summarized according to the closest reported relationship.														

Table 12: Summary of Clinical Laboratory Results by Parameter, Maximum Severity, Visit, and Vaccination Group – [Parameter]

Visit	Vaccination Group	N	None		Mild / Grade 1		Moderate/ Grade 2		Severe/ Grade 3		Potentially Life Threatening/ Grade 4		Missing	
			n	%	n	%	n	%	n	%	n	%	n	%
Baseline (Day 0)	200 µg ALFQ	x	x	x.x	x	x.x	x	x.x	x	x.x	x	x.x	x	x.x
	100 µg ALFQ	x	x	x.x	x	x.x	x	x.x	x	x.x	x	x.x	x	x.x
	50 µg ALFQ	x	x	x.x	x	x.x	x	x.x	x	x.x	x	x.x	x	x.x
[Additional visits]	200 µg ALFQ	x	x	x.x	x	x.x	x	x.x	x	x.x	x	x.x	x	x.x
	100 µg ALFQ	x	x	x.x	x	x.x	x	x.x	x	x.x	x	x.x	x	x.x
	50 µg ALFQ	x	x	x.x	x	x.x	x	x.x	x	x.x	x	x.x	x	x.x
Max Severity Post Baseline	200 µg ALFQ	x	x	x.x	x	x.x	x	x.x	x	x.x	x	x.x	x	x.x
	100 µg ALFQ	x	x	x.x	x	x.x	x	x.x	x	x.x	x	x.x	x	x.x
	50 µg ALFQ	x	x	x.x	x	x.x	x	x.x	x	x.x	x	x.x	x	x.x

Note: The "Max Post Baseline" rows indicate the maximum severity experienced by each participant at any time point post baseline, including unscheduled assessments. N = Number of participants in the Safety Population with available samples; n = Number of participants reporting each severity

Table 13: Clinical Laboratory Summary Statistics by Visit and Vaccination Group – [Parameter]

Time Point	Vaccination Group	N	Mean	Standard Deviation	Median	Min, Max
Baseline (Day 0)	200 µg ALFQ	x	xx.x	xx.x	xx.x	xx.x, xx.x
	100 µg ALFQ	x	xx.x	xx.x	xx.x	xx.x, xx.x
	50 µg ALFQ	x	xx.x	xx.x	xx.x	xx.x, xx.x
[Additional visits]	200 µg ALFQ	x	xx.x	xx.x	xx.x	xx.x, xx.x
	100 µg ALFQ	x	xx.x	xx.x	xx.x	xx.x, xx.x
	50 µg ALFQ	x	xx.x	xx.x	xx.x	xx.x, xx.x
[Additional visits, Fold Change from Baseline]	200 µg ALFQ	x	xx.x	xx.x	xx.x	xx.x, xx.x
	100 µg ALFQ	x	xx.x	xx.x	xx.x	xx.x, xx.x
	50 µg ALFQ	x	xx.x	xx.x	xx.x	xx.x, xx.x
Note: N = Number of participants in the Safety Population with results available						

IMMUNOGENICITY

Table 14: Durability of Immunology Response as Measured by Area Under the Response Curve, by Antigen and Vaccination Group – [Assay name]

	200 µg ALFQ (N=X)		100 µg ALFQ (N=X)		50 µg ALFQ (N=X)		
Antigen	**n**	**Mean (95% CI)**	**n**	**Mean (95% CI)**	**n**	**Mean (95% CI)**	**p-value[a]**
gp120 A244gD-D11	xx	xx.x (xx.x, xx.x)	xx	xx.x (xx.x, xx.x)	xx	xx.x (xx.x, xx.x)	0.xx
gp120 MNgD- D11	xx	xx.x (xx.x, xx.x)	xx	xx.x (xx.x, xx.x)	xx	xx.x (xx.x, xx.x)	0.xx
[Additional antigens]							

Note: N = Number of participants in the Immunogenicity Population; n = Number of participants with available analysis values ·**ANOVA used to compare mean log AUC across all vaccination groups**

Table 15: Immunology Geometric Mean Titers and Associated 95% CIs by Visit, Antigen, and Vaccination Group – [assay name]

		200 µg ALFQ (N=X)		100 µg ALFQ (N=X)		50 µg ALFQ (N=X)		
Visit	**Antigen**	**n**	**GMT (95% CI)**	**n**	**GMT (95% CI)**	**n**	**GMT (95% CI)**	**p-value**
Baseline (Day 0)	gp120A244gD-D11	xx	xx.x (xx.x, xx.x)	xx	xx.x (xx.x, xx.x)	xx	xx.x (xx.x, xx.x)	0.xx
	[Additional antigens]	xx	xx.x (xx.x, xx.x)	xx	xx.x (xx.x, xx.x)	xx	xx.x (xx.x, xx.x)	0.xx
		xx	xx.x (xx.x, xx.x)	xx	xx.x (xx.x, xx.x)	xx	xx.x (xx.x, xx.x)	0.xx
[Additional visits or Peak]		xx	xx.x (xx.x, xx.x)	xx	xx.x (xx.x, xx.x)	Xx	xx.x (xx.x, xx.x)	0.xx

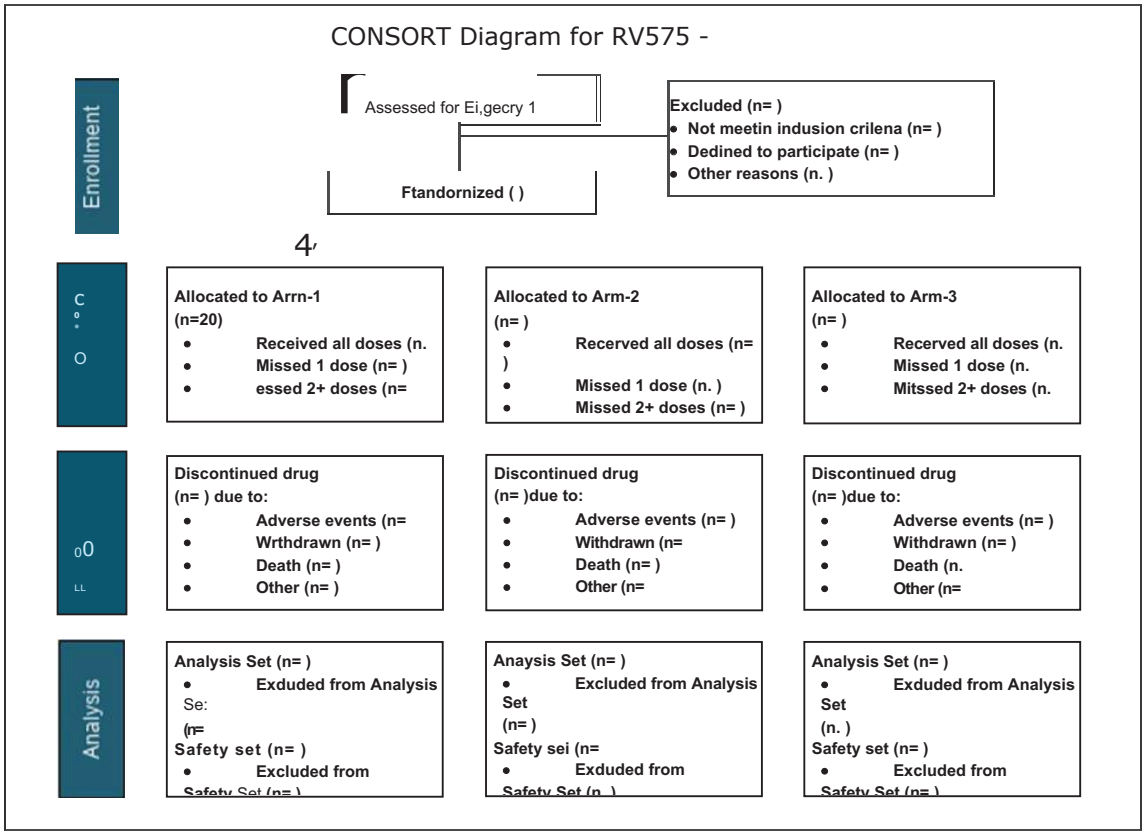
Note: This will be adapted for assays with continuous analysis values, as appropriate

Table 16: Geometric Mean Titers and Associated 95% CIs by Visit, Antigen, and Vaccination Group – [assay name]

Visit	Antigen	200 µg ALFQ (N=X)		100 µg ALFQ (N=X)		50 µg ALFQ (N=X)		p-value[a]
		n	GMT (95% CI)	n	GMT (95% CI)	n	GMT (95% CI)	
Baseline (Day 0)	CD4+ T cells all IFNγ+	xx	xx.x (xx.x, xx.x)	xx	xx.x (xx.x, xx.x)	xx	xx.x (xx.x, xx.x)	0.xx
	CD4+ T cells all IL2+	xx	xx.x (xx.x, xx.x)	xx	xx.x (xx.x, xx.x)	xx	xx.x (xx.x, xx.x)	0.xx
	CD4+ T cells all IFNγ and IL2	xx	xx.x (xx.x, xx.x)	xx	xx.x (xx.x, xx.x)	xx	xx.x (xx.x, xx.x)	0.xx
	CD4+ T cells all IFNγ+ or IL2+	xx	xx.x (xx.x, xx.x)	xx	xx.x (xx.x, xx.x)	xx	xx.x (xx.x, xx.x)	
	CD8+ T cells all IFNγ+	xx	xx.x (xx.x, xx.x)	xx	xx.x (xx.x, xx.x)	xx	xx.x (xx.x, xx.x)	
	CD8+ T cells all IL2+	xx	xx.x (xx.x, xx.x)	xx	xx.x (xx.x, xx.x)	xx	xx.x (xx.x, xx.x)	
	CD8+ T cells all IFNγ and IL2	xx	xx.x (xx.x, xx.x)	xx	xx.x (xx.x, xx.x)	xx	xx.x (xx.x, xx.x)	
	CD8+ T cells all IFNγ+ or IL2+	xx	xx.x (xx.x, xx.x)	xx	xx.x (xx.x, xx.x)	xx	xx.x (xx.x, xx.x)	
[Additional visits or Peak]		xx	xx.x (xx.x, xx.x)	xx	xx.x (xx.x, xx.x)	xx	xx.x (xx.x, xx.x)	0.xx

Note: This will be adapted for assays with continuous analysis values, as appropriate

Figure 1: CONSORT Flow Diagram



Listing 1: Participants Receiving Study Vaccination

Participant ID	Study Group	Date of Study Vaccine Administration	Date of Study Vaccine Administration	Date of Study Vaccine Administration

Listing 2: Discontinuations and Early Terminations

Participant ID	Study Day of Discontinuation	Reason for Discontinuation	If Due to AE, AE Number	Terminated from Study?

Listing 3: Participants Not Receiving Assigned Study Vaccination

Participant ID	Date of Enrollment	Assigned Study Group	Actual Study Group

Listing 4: Protocol Deviations

Participant ID	Study Group	Deviation Number	Deviation	Study Day
[N/A if screening failure]				

Listing 5: Prior and/or Concurrent Medical Conditions Meeting Exclusion Criteria

Participant ID	Study Group	Exclusion Criterion	Study Day Criterion Met	Body System	Preferred Term
	[N/A if screening failure]		[N/A if screening failure]		

Listing 6: Prior and Concomitant Medications Meeting Exclusion Criteria

Participant ID	Study Group	Exclusion Criteria	Study Day Criterion(a) Met	Medication Name
	[N/A if screening failure]		[N/A if screening failure]	

Listing 7: Analysis Populations

Participant ID	Study Group	Analysis Excluded

Listing 8: Incident HIV Infections

Participant ID	Study Group	Study Day of Reported HIV Infection	Comments

Listing 9: Serious Adverse Events

Participant ID	Study Group	Adverse Event Number	Adverse Event	Study Day Reported	Duration	Number of Days Post-Vaccination the Event Became Serious	Reason Reported as an SAE	Severity	Relationship to Study Vaccination	If Not Related, Alternate Etiology	Study Vaccine Change	Resolution	Comments

Listing 10: Non-Serious, Related Unsolicited Adverse Events

Participant ID	Study Group	Adverse Event Number	Adverse Event	Study Day Reported	Duration	Severity	Relationship to Study Vaccine	Study Vaccine Change	Resolution	Comments

Listing 11: Clinical Laboratory Values

Participant ID	Study Group	Visit	Actual Study Day	Sex	Age (years)	Laboratory Parameter (Units)	Result (Severity Grade)	Reference Range Low	Reference Range High
						[Units may be +/- or N/A for some lab tests]			

Listing 12: Pregnancies

Participant ID	Study Group	Pregnancy Outcome	Date of Delivery	Date of Termination	If Terminated, Due to a Verified Congenital Anomaly?	Infant Sex	Infant Weight at Birth (g)	Estimated Gestational Age of Infant at Birth (weeks)	Was the Infant Born with Congenital Anomalies?
				[N/A for deliveries]					